WEST Search History

DATE: Tuesday, October 29, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L11	L10 not l6	15	L11
L10	l1 and l5	22	L10
L9	L8 not l6	15	L9
L8	l3 and l5	22	L8
L7	3817830.pn.	4	L7
L6	l4 and l5	7	L6
L5	putida	1922	L5
L4	l1 same l2	36	L4
L3	l1 and L2	249	L3
L2	Pseudomonas	28236	L2
L1	vanilli$	6384	L1

END OF SEARCH HISTORY

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L6: Entry 3 of 7 File: USPT Jan 18, 1994

DOCUMENT-IDENTIFIER: US 5279950 A
TITLE: Bioconversion process for the production of vanillin

Brief Summary Text (5):
It is well known that vanillin is formed in small quantities from aromatic compounds known to be precursors in the biosynthesis of lignin (see Rahouti, Mohammed, et al., Appln. Environ. Microbiol., 55:2391-8, Sep., 1989; Tadasa, K., Agric. Biol. Chem. 41:925-9, 1977; Sutherland, J. B. et al., Can. J. Microb., 29:1253-7, 1983). Trans-ferulic is a common precursor in the biosynthesis of lignin as well as a lignin degradation product. The metabolism of ferulic acid by soil microorganisms typically leads to the production of vanillin but the vanillin so formed is further converted to vanillic acid and/or vanillyl alcohol which in turn are typically transformed to other degradation products such as protocatechuic acid, guaiacol, hydroquinones, catechol, ring-cleavage products, etc. Thus, it is generally recognized that vanillin is an intermediate in the overall scheme for the biodegradation of ferulic acid and ferulic acid precursors so that only small amounts of vanillin accumulate when ferulic acid is biodegraded by soil microorganisms. There are a large number of references which report on the metabolism of aromatic compounds by soil microorganisms (e.g., Pullularia pullulans Fusarium sp., Aspergillus niger, Bacillus sp., Pseudomonas sp., Nocardia sp., see Borneman, W. S. et al., Appl. Microbiol. Biotechnol. 33:354-51, 1990; Eggeling, L. et al., Arch. Microbiol. 126:141-8, 1980) but none stress the potential for using such microorganisms for vanillin synthesis because vanillin accumulates only small quantities as an intermediate product.

Brief Summary Text (14):
As a source of ferulic acid degrading microorganism, any of the common types cited in the literature capable of the degradation of ferulic acid may be used provided the microorganism is able to effect the degradation through a pathway wherein vanillin is an intermediate. Thus, it is envisaged that many types of fungi, bacteria and yeasts will work in the present invention as a large number of microorganisms with this capability are known. For the purposes of this invention, "ferulic acid degrading microorganism" will hereinafter refer to those microorganisms capable of growth on trans-ferulic acid to 4-substituted guaiacols by a pathway wherein vanillin is an intermediate. Illustrative of this (but not limiting) are Paecilomyces varioti and Pestaloti palmarum which convert ferulic acid to vanillin via 4-vinylguaiacol (see Rahouti, M. et al. above) as do Pseudomonas cepacia (see Andreoni, V., et al., Syst. Appl. Microbiol., 5:299-304, 1980), and Fusarium solani (see Nazareth, S. et al., Can. J. Microbiol., 32:494-7, 1986). Polyporous versicolor (Ishikawa, H., et al., Arch. Biochem. Biophys., 100:140-9, 1963), and Pseudomonas acidovorans (Toms, A., et al., Biochemistry 9:337-43, 1970) are also known to transform ferulic acid to vanillin via elimination of side chain carbons. A Corynebacteriumg sp. is capable of transforming eugenol to vanillin via ferulic acid (Tadasa, K., Agric. Biol. Chem., 41(6):925-9, 1977).

Brief Summary Paragraph Table (2):

Microorganism	Precursor	Vanillin Conc. (ug/ml) DTT	Vanillin DTT	Without	With
Aspergillus niger	Ferulic Acid	2.2	93.7	"	
	Eugenol	1.2	18.5		
Pseudomonas putida	Ferulic Acid	0.4	139.4	"	
	Eugenol	1.2	4.7		
Corynebacterium glutamicum	Ferulic Acid	16.2	115.0	"	
	Eugenol	3.3	3.8		
Rhodotorula glutinis	Ferulic Acid	0.4	80.6		

Detailed Description Text (2):
This example illustrates the effect of a sulfhydryl agent (dithiothreitol) in

increasing the production of vanillin when vanillin precursors are treated with Pseudomonas putida cells.

Detailed Description Text (3):
Pseudomonas putida ATCC 55180 was inoculated into 50 ml of Mandels medium containing 1% glucose and was cultured at 30 degrees C. for 16 hours on a rotary shaker. The resulting cells were harvested by centrifugation and were resuspended in 10 ml of Mandels medium with no added glucose. One-ml portions of the cell suspension were added to 9-ml portions of four different reaction mixtures to form the bioconversion mixtures shown in Table 1. The mixtures were incubated at room temperature (ca. 25 degrees C.) without aeration. Vanillin concentration was determined in the mixtures at the indicated times. All bioconversion mixtures contained 0.1% glucose in addition to the added cells and reactants shown in the Table.

Detailed Description Text (9):
This example shows the bioconversion of vanillin precursors in the presence of dithiothreitol using Pseudomonas acidovorans ATCC 15668.

Detailed Description Text (13):
This example shows bioconversion of ferulic acid by Pseudomonas putida in the presence of different sulfhydryl containing compounds.

Detailed Description Text (14):
Pseudomonas putida ATCC 55180 was inoculated into 100 ml fresh YNB medium containing 1% glucose. After culturing for 7 hours at 30 degrees C. with shaking at 250 rpm, the cells were collected and transferred into 200 ml of fresh YNB medium containing 1% glucose and cultured overnight on a rotary shaker at 30 degrees C. Cells were collected by centrifugation for 10 min. at 10,000 rpm and were used to form four different 10-ml bioconversion mixtures containing Mandels medium, 0.1% glucose and 0.1% FA. Each mixture contained a different sulfhydryl compound (concentration 10 mM) as shown in Table 4 below. The mixtures were incubated for varying periods of time and vanillin concentration determined.

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L6: Entry 1 of 7 File: USPT Nov 27, 2001

DOCUMENT-IDENTIFIER: US 6323011 B1
TITLE: Production of vanillin

Abstract Text (1):
A method of producing vanillin comprising the steps of: (1) providing trans-ferulic acid or salt thereof; and (2) providing trans-ferulate; CoASH ligase activity (enzyme activity I) trans-feruloyl SCoA hydratase activity (enzyme activity II), and 4-hydroxy-3-methoxyphenyl-.beta.-hydroxy-propionyl SCoA (HMPHP SCoA) cleavage activity (enzyme activity III). Conveniently the enzymes are provided by Pseudomonas fluorescens Fe3 or a mutant or derivative thereof. Polypeptides with enzymes activities II and III and polynucleotides encoding said polypeptides. Use of said polypeptides or said polynucleotides in a method for producing vanillin.

Brief Summary Text (4):
In the Gram-negative bacterium, Pseudomonas acidovorans, trans-ferulate was shown to be catabolised to vanillate and acetate, apparently via vanillin (A. Toms and J. M. Wood, Biochemistry 9, 337-343 (1970)). Although in cell-free extracts NAD.sup.+ was necessary for the oxidation of vanillin to vanillate and for the further oxidation of vanillate to protocatechuate and formate, no mention was made of any other cofactor requirements. Further studies of ferulate utilisation in Pseudomonas species have been reported (V. Andreoni and G. Bestetti, FEMS Microbiology Ecology 53, 129-132 (1988); T. Omori, K. Hatakeyama and T. Kodama, Appl. Microbiol. Biotechnol. 29, 497-500 (1988); Z. Huang, L. Dostal and J. P. N. Rosazza, Appl. Env. Microbiol. 59, 2244-2250 (1993)); however, these have not sought to elucidate further the mechanism of the two-carbon cleavage of ferulate. Zenk et al (1980) Anal. Biochem. 101, 182-187 describe a procedure for the enzymatic synthesis and isolation of cinnamoyl-CoA thioesters using a bacterial system. In contrast, the enzymology and genetics of the utilisation of simple benzene derivatives, including benzoic acids and phenols, by Pseudomonas have been intensively studied (T. K. Kirk, T. Higuchi and H.-M. Chang (eds.), "Lignin biodegradation", CRC Press, Boca Raton, Fla., USA (1980); D. T. Gibson (ed.), "Microbial degradation of organic compounds", Marcel Dekker, New York (1984); J. L. Ramos, A. Wasserfallen, K. Rose and K. N. Timmis, Science 235, 593-596 (1987); C. S. Harwood, N. N. Nichols, M. K. Kim, J. L. Ditty and R. E. Parales, J. Bacteriol. 176, 6479-6488 (1994); S. Romerosteiner, R. E. Parales, C. S. Harwood and J. E. Houghton, J. Bacteriol. 176, 5771-5779 (1994); J. Inoue, J. P. Shaw, M. Rekik and S. Harayama, J. Bacteriol. 177, 1196-1201 (1995;)).

Brief Summary Text (16):
We have determined the mechanism of chain-shortening of trans-ferulate (trans-ferulic acid) by a strain of Pseudomonas fluorescens (named Ps. fluorescens biovar. V, strain AN103 and which we have abbreviated at some points to AN103) isolated from soil. Our data indicate clearly that vanillin (4-hydroxy-3-methoxy benzaldehyde) is an intermediate and that the mechanism does not involve .beta.-oxidation. The vanillin pathway of Ps. fluorescens biovar. V, strain AN103 is described in FIG. 1. Trans-ferulic acid (or a salt thereof) is interconverted with trans-feruloyl SCoA in the presence of CoASH; trans-feruloyl SCoA is interconverted with 4-hydroxy-3-methoxyphenyl-.beta.-hydroxypropionyl SCoA (HMPHP SCoA); and HMPHP SCoA is interconverted with vanillin.

Brief Summary Text (21):
It is preferred if means for converting vanillin to a non-vanillin product is absent or reduced. Of course, the enzyme activity III is not such a means. Conveniently, these enzyme activities are provided by the soil bacterium Pseudomonas fluorescens biovar. V, strain AN103 the said bacterium being that deposited under the Budapest

Treaty at the National Collection of Industrial and Marine Bacteria Limited, AURIS Business Centre, 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland under Accession No NCIMB 40783, or a mutant or variant thereof. By "mutant or variant thereof" we include any mutant or variant of the said bacterium provided that the bacterium retains the said enzyme activities whether or not at the same levels. It will be appreciated that the said enzyme activities can be retained even if the genes encoding said enzymes are mutated. For example, mutants which constitutively express (as opposed to conditionally or inducibly express) the said enzyme activities are particularly useful mutants of Ps. fluorescens biovar. V, strain AN103, as are variants in which one or more of the enzymes with the said activities exhibit more favourable kinetic characteristics (for example, an increased turnover number or a decreased K.sub.m).

Brief Summary Text (74):
A third aspect of the invention provides Pseudomonas fluorescens biovar. V, strain AN103 as deposited under the Budapest Treaty at the National Collections of Industrial and Marine Bacteria Limited, AURIS Business Centre, 23 St Machar Drive, Aberdeen AB2 1RY, Scotland under Accession No NCIMB 40783, or a mutant or variant thereof. Preferred mutants and variants are the same as those preferred in the first aspect of the invention. A particularly preferred mutant of Ps. fluorescens biovar. V, strain AN103 is one which accumulates vanillin when provided with trans-ferulic acid or a salt thereof. Conveniently, this is a mutant of Ps. fluorescens biovar. V, strain AN103 wherein vanillin:NAD.sup.+ oxidoreductase activity is absent or reduced. Suitably, there is a mutation in the gene encoding vanillin:NAD.sup.+ oxidoreductase such that the enzyme activity is absent or substantially reduced. Such a mutant can be made as described above.

Brief Summary Text (164):
Typically, in a biochemical process the strain of Pseudomonas (eg Ps. fluorescens biovar. V, strain AN103) provides an enzyme system for the biotransformation of plant derived trans-ferulic acid to vanillin and/or related compounds. Enzyme preparations, whole cells of Pseudomonas or a heterologous host organism expressing appropriate Pseudomonas genes may be used for this. A variety of mutants of Pseudomonas and various additional enzyme preparations, co-factors or co-factor regenerating systems may be used. The Pseudomonas enzymes might be overexpressed in a heterologous host before being extracted and used in a biotransformation.

Brief Summary Text (165):
Alternatively, but suitably, some form of fermentation process may be used which involves the Pseudomonas strain or an appropriate derived mutant or a heterologous host organism in which the genes for biotransformation are expressed. The chosen microorganism is typically grown on a ferulate-rich substrate or a substrate comprising trans-feruloyl SCoA. This could generate a vanillin production process.

Brief Summary Text (172):
Still further aspects of the invention provide use of Pseudomonas fluorescens biovar. V, strain AN103 or a mutant or derivative thereof in a method for producing vanillin, or vanillic acid or salt thereof; use of a polypeptide of the invention in a method for producing vanillin, or vanillic acid or salt thereof; use of a polynucleotide of the invention in a method for producing vanillin, or vanillic acid or salt thereof; and use of a host cell of the invention in a method for producing vanillin or vanillic acid or a salt thereof.

Drawing Description Text (3):
FIG. 1 describes the vanillin pathway in Pseudomonas fluorescens biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-.beta.-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-ferulic acid and trans-feruloyl SCoA; II is an enzyme that catalyses the interconversion of trans-feruloyl SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and vanillin; and IV is an enzyme that catalyses the interconversion of vanillin and vanillic acid.

Detailed Description Text (15):
The organism was isolated from soil samples rich in decayed vegetation and was shown to be a strain of Pseudomonas fluorescens using standard identification techniques.

As shown in Table I, the bacterium would grow not only on trans-ferulate as sole carbon source, but also on several closely-related compounds, including vanillate, protocatechuate and caffeate. Growth on vanillin was observed at low concentrations (<1 MM) but was variable; higher concentrations were growth-inhibitory. If the organism was grown on vanillate, transfer to medium containing transferulate as sole carbon source was followed by a lag in the growth curve; this was not observed if the transfer was to medium containing both vanillate and trans-ferulate (FIG. 2). During a growth cycle on trans-ferulate, a transient increase in vanillate was observed at around the time when trans-ferulate disappearance was maximal (FIG. 3), suggesting that vanillate was a catabolite of trans-ferulate. A small amount of protocatechuate was also observed when the culture medium was examined by TLC (not shown).

Detailed Description Text (79):
Isolation of the Genes Required for the Conversion of Trans-feruloyl SCoA to Vanillic Acid (Vanillate) in Pseudomonas fluorescens Strain AN103

Detailed Description Text (80):
A strain of Pseudomonas fluorescens (biovar. V, AN103) was isolated from soil at the Institute of Food Research, Norwich Laboratory, which was able to grow on trans-ferulic acid converting it to vanillic acid via vanillin. The proposed biochemical pathway for the conversion of transferulic acid to vanillic acid shown in FIG. 1 was substantiated in the experiments described above in Experiments 2-4.

Detailed Description Text (89):
The presence of a novel enzyme activity (cf. Example 4) in the E. coli clone was demonstrated. E. coli cells were grown at 37.degree. C. for 3 h in 50 ml of L medium, containing ampicillin (50 .mu.g/ml) with and without induction by IPTG. Extracts were prepared as described above in Example 2 for Ps. fluorescens AN103, but without centrifugation. The crude extract was used for assay. Enzyme activity with both HMPHP SCoA and trans-feruloyl ScoA was determined as described above in Example 4 using HPLC to determine the reaction products. The results presented in Table IX clearly demonstrate that vanillin and acetyl SCoA were produced in equimolar proportions both with trans-feruloyl SCoA and with HMPHP SCoA as substrates. In addition, HMPHP SCoA was also dehydrated to feruloyl SCoA, putatively trans-feruloyl SCoA, demonstrating the reverse of activity II. These results are closely similar to those obtained with the vanillin-forming cleavage enzyme purified from Pseudomonas fluorescens AN103, although the ratio of activities with trans-feruloyl SCoA and HMPHP SCoA differs slightly between the two preparations. There was no activity with either trans-feruloyl SCoA or HMPHP SCoA in extracts of an unmanipulated E. coli strain, whether induced or not. In the manipulated strain E. coli 1039 that expresses the Pseudomonas gene the specific activity was slightly lower in the extract made following induction than in that made from uninduced bacteria. Since the assay measures only active enzyme it is conceivable that increased protein expression occurs upon induction but this may result in incorrectly folded and therefore inactive enzyme. It was not possible to detect expression of the 31 kD protein visually on Coomassie-stained, one-dimensional SDS gels because of its co-migration with the strongly expressed .beta.-lactamase encoded by the vector ampicillin resistance marker.

Other Reference Publication (5):
Bare et al., "Bioconversion of Vanillin into Vanillic Acid by Pseudomonas fluorescens Strain BTP9," Appl. Biochem. Biotech., 45/46:599-610 (1994).

Other Reference Publication (26):
Romero-Steiner et al., "Characterization of the pcaR Regulatory Gene from Pseudomonas putida, Which is Required for the Complete Degradation of p-Hydroxybenzoate," J. Bacteriol., 176:5771-5779 (1994).

Other Reference Publication (27):
Inoue et al., "Overlapping Substrate Specificities of Benzaldehyde Dehydrogenase (the xylC Gene Product) and 2-Hydroxymuconic Semialdehyde Dehydrogenase (the xylG Gene Product) Encoded by TOL Plasmid pWW0 of Pseudomonas putida," J. Bacteriol., 177:1196-1201 (1995).

Other Reference Publication (52):
Lute, "The Pathway of Phenylpropanoid Degradation in Pseudomonas putida mt-2," (Ph.D. Thesis).